AD_____

Award Number: DAMD17-00-1-0480

TITLE: Development of Laulimalide-Based Microtubule-Stabilizing Agents: New Chemistry for the Treatment of Breast Cancer

PRINCIPAL INVESTIGATOR: Bradley S. Davidson, Ph.D.

CONTRACTING ORGANIZATION: Utah State University
Logan, Utah 84322-1415

REPORT DATE: July 2003

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	July 2003	Annual (1 Jul 02-30 Jun 03)

4. TITLE AND SUBTITLE
Development of Laulimalide-Based Microtubule-Stabilizing Agents: New Chemistry for the Treatment of Breast Cancer

5. FUNDING NUMBERS
DAMD17-00-1-0480

6. AUTHOR(S)
Bradley S. Davidson, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Utah State University
Logan, Utah 84322-1415

E-Mail: davidson@cc.usu.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 Words)

Through a collaborative research program involving Utah State University and the Cancer Research Center of Hawaii, we have discovered that the sponge-derived macrolides laulimalide and isolaulimalide are potent cytotoxins with paclitaxel-like antimicrotubule-stabilizing activity. Laulimalide is a potent inhibitor of cellular proliferation with an IC_{50} in the low nanomolar range and it maintains activity against a drug resistant, P-glycoprotein over-expressing ovarian cancer cell line. Laulimalide represents a lead compound for new class of microtubule-stabilizing agents with activities that may prove therapeutically useful for the treatment of breast cancer. The aim of this project is to utilize a combinatorial solid-phase synthetic approach for the construction of a library of laulimalide analogs for structure activity relationship (SAR) studies.

In an effort to discover a new chemotherapeutic agent for the treatment of breast cancer we propose to do the following: 1) transfer our current solution-phase synthetic approach to solid phase, 2) using a split and pool strategy, prepare 260 laulimalide analogs, 3) test laulimalide analogs for microtubule-stabilizing activity, cytotoxicity against both drug-sensitive and drug-resistant breast cell lines, and 4) submit active analogs to the NCI for screening in the 60-cell line.

Work to-date has been concentrated on the adaptation of our solution phase laulimalide synthetic methods for efficient conversion to the solid phase. Because of difficulties adding fragment **C**, a new retrosynthetic approach has been developed and is now being explored. It has the advantage that the coupling of fragment **C** to the resin-bound material will not involve the formation of a new asymmetric center, thus simplifying the reaction. Fragment **B** analogs have been prepared and coupled using a Juliea-Kocienski type reaction to an appropriate aldehyde and the first two laulimalide analogs have been screened for biological activity..

14. SUBJECT TERMS
No subject terms provided.

15. NUMBER OF PAGES
8

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

Table of Contents

Introduction

In 1999 we reported that the marine macrolides laulimalide (**1**) is a new Taxol-like microtubule-stabilizing agents with activity against drug resistant cancer cells,[1] It therefore represents a new class of microtubule-stabilizing agent with activities that may prove therapeutically useful. The purpose of the proposed study is to produce an effective new agent for the treatment of breast cancer. With the goal of discovering a new chemotherapeutic agent for the treatment of breast cancer, we proposed to do the following: (a) transfer our current solution-phase synthetic technology to the solid phase; (b) using a split-and-pool strategy, prepare a library of up to 260 laulimalide analogs that are designed for 1) improved stability, 2) ease of synthesis, and 3) structural diversity; (c) screen laulimalide analogs for tubulin polymerization promoting activity and cytotoxicity toward drug-sensitive and drug-resistant breast cancer cell lines; and (d) submit active analogs to the NCI for screening in the 60-cell line assay Our future goals include the selection of the most promising analogs for scale-up synthesis and *in vivo* testing.

Body

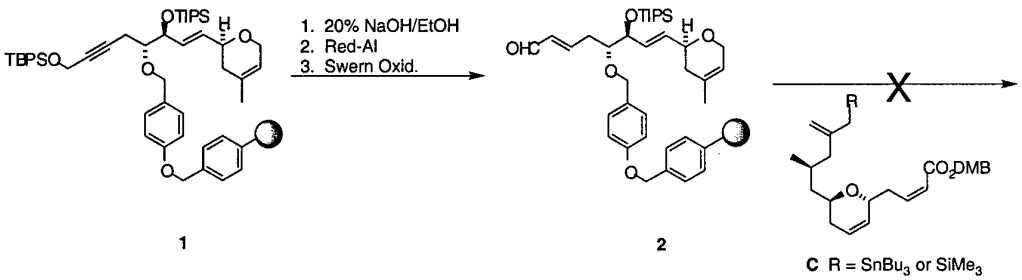
Research accomplishments for year 3 are described below for each of the Tasks in the approved Statement of Work.

Task 1. To adapt solution-phase synthetic technology to use on the solid phase (months 1-12)

- Establish conditions for linking compounds to resin and for cleaving products from resin
- Optimize reaction times/conditions for solid phase, starting with conditions established for solution phase
- Synthesize laulimalide on solid phase

Task 1 is, unfortunately, still consuming much of our effort. Two crucial steps have presented difficulties: 1) the conversion of compound **1** to **2** (**10** to **11** in year 2 report), which been accomplished only in low yields and 2) the addition of major fragment **C** to **2** (represented in scheme 1 for the solid-phase) could not be accomplished in solution under conditions that would be sufficiently robust and generally applicable for the use on solid phase with a variety of analog fragments. The difficulty with the conversion from **1** to **2** results from insufficient stability of the linker to the conditions needed for removal of the *tert*-butyldiphenylsilyl (TBPS) protecting group. While this step works without difficulty in the solution phase, it has been unsatisfactory when applied to the solid phase synthesis. Because the TBPS group proved inert to the NaOH/MeOH, conditions that readily remove it in solution, we made two modifications. First we substituted the more reactive KOH for NaOH and we substituted EtOH for MeOH, which improved the swelling of the resin. Unfortunately, the reduction of the propargyl alcohol to the allylic alcohol with Red-Al was also problematic.

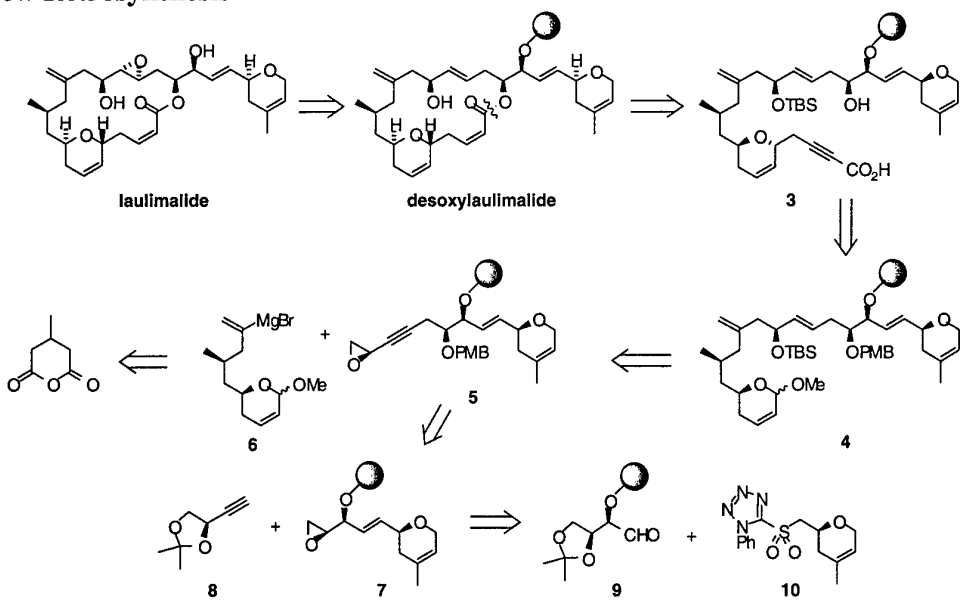
Scheme 1.



The asymmetric allylation reaction that we proposed for the addition of fragment **C** to **2** has presented challenges to several research groups.[2] While methods have been developed, all involve highly sensitive, tricky reactions that are not likely to be amenable to solid-phase combinatorial synthesis. For these reasons, our project has evolved from trying to simply adapt out solution-phase synthesis to the solid-phase to having to drastically modify our approach. However, we feel that our new strategy will provide solutions to the above problems and lead to a stronger, more robust methodology.

Because we view the biggest challenge to be the addition of fragment **C**, we have devised a simpler approach, involving the opening of an epoxide ring with a Grignard reagent (**6** + **5**; see Scheme 2). This approach has the advantage that it does not include the formation of a new chiral center, with the C_{15} center coming from ascorbic acid, via compound **8**. Further changes include: 1) we will attach fragment **B** (side chain; **10** below) before adding the C_{14}-C_{17} fragment (**8**), using methodology that we have developed, an approach that allows us to eliminate some protecting group chemistry, and 2) we have decided to adopt the ynoate chemistry, first demonstrated by Ghosh,[3] for the macrocyclization, as it allows the formation of the labile (*Z*)-α,β-unsaturated ester to be done very late in the synthesis. With the new approach the C_{19} alcohol is not available until after the addition of fragment **B**, one of the diversity generating pieces; therefore, we have moved our resin attachment point to the C_{20} alcohol. This modification also has the benefit of, ultimately, allowing the macrolactonization reaction to be undertaken while the molecule is still bound to the resin, in contrast to our previous approach, which required several steps to be carried out after cleavage, a shortcoming when we begin library synthesis.

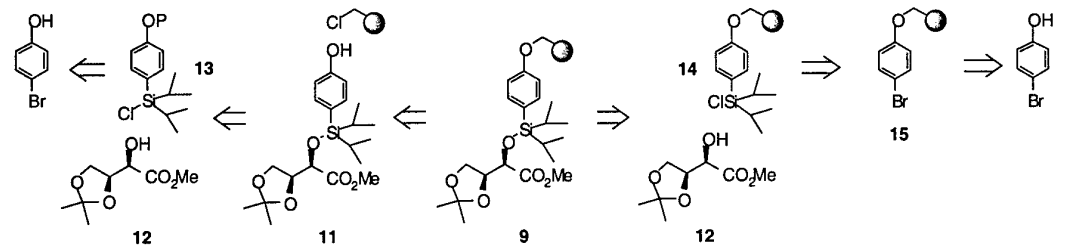
Scheme 2. New Retrosynthesis



We have been developing a new method for attaching our substrate to the solid-phase resin. Because we had previously been protecting the C_{20} alcohol with a silyl group, we are preparing a new silyl linker. Instead of the TIPS protecting group that we have used thus far, we are preparing a diisopropylphenylsilyl analog, where the phenyl group will link to the resin through a phenol. The silyl chloride is formed from the silane with trichloroisocyanuric acid and the silane is formed by the addition of an aryllithium species to chlorodiisopropylsilane. We have prepared compound **11** protected with a PMB ether, but had difficulty removing the PMB group. With a methylthiomethyl (MTM) protecting proup the conversion from a silane

to the silyl chloride was unsuccessful. We are not trying a *tert*-butyldimethylsilyl group, with the hope of selectively removing it in the presence of the silyl linker. Alternatively, The linker could be prepared on the resin from **14** and **15**.

Scheme 3. Linker Synthesis.

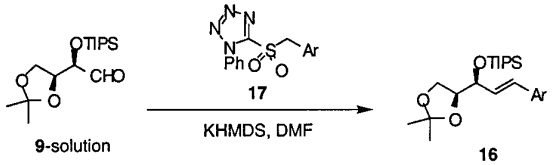


Task 2. Using a split-and-pool strategy, prepare a library of up to 260 laulimalide analogs that are designed for 1) improved stability, 2) ease of synthesis, and 3) structural diversity.

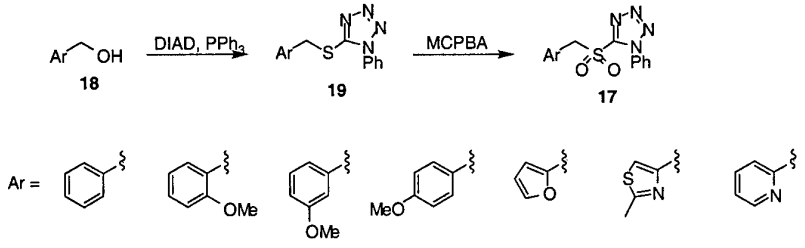
- Synthesize subunits **A2, B1-B10, C1-C15.** (months 1-12)
- Adapt solid phase reactions for use in micro-reactors (months 6-12)
- Phase I combinatorial synthesis of 60 laulimalide analogs (months 13-18)
- Phase II combinatorial synthesis of 200 laulimalide analogs (months 24-36)

Subunit synthesis has continued during year 3. While we had previously prepared **B** subunits as phosphonate esters with the goal of gaining the desired side chains with a *trans* geometry, attempts to carry out the Horner-Emmons type of reaction were unsuccessful under using a wide range of bases and solvents, often leading to decomposition of the aldehyde. Therefore, we renewed our attempts to prepare the sulfone reagents to be used in Julia-Kocienski reactions (see Scheme 4), analogous to our solution-phase work. To this effect, arylmethyl alcohols were coupled with 1-phenyl-5-mercaptotetrazole under Mitsunobu conditions to give sulfides that need to be oxidized to the corresponding sulfones. Previously, attempts to carry out the oxidation using a variety of reagents, including ammonium molybdate-H_2O_2, H_2O_2-MeOH, oxone, and magnesium monoperphthalate, gave only low yields using a variety of oxidizing agents. Fortunately, oxidation with *m*-chloroperoxybenzoic acid cleanly provides the desired sulfones, facilitating the preparation of the necessary reagents (Scheme 5).

Scheme 4. Propose Strategy for Addition of Side Chain.

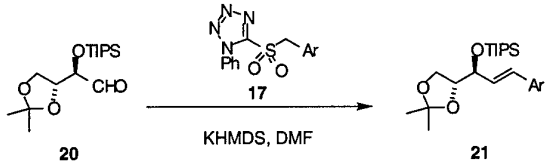


Scheme 5. Preparation of Sulfones.



Once prepared, coupling of the sulphone reagents **17** with aldehyde **20** yielded the corresponding products **21**. For Ar = Ph, a 74% yield was obtained, with a 5:1 ratio of *trans*:*cis*. The reactions were similarly successful with the methyoxyphenyl analogs and with the furfuryl derivative, however, the thiazole and pyridine analogs have not yet been prepared.

Scheme 6.



20 **21**

Task 3. Screen laulimalide analogs for tubulin polymerization promoting activity and cytotoxicity toward drug-sensitive and drug-resistant breast cancer cell lines.

- Establish benchmark activities for laulimalide in assays (months 13-18)
- Test Phase I combinatorial library (months 19-24)
- Test Phase II combinatorial library (months 25-36)

The first laulimalide analogs prepared as part of this study (**22** and **23**) were submitted to Dr. Mooberry for biological testing, but were shown to be inactive.

22 R = *p*-nitrobenzoate **23**

Task 4. Submit active analogs to the NCI for screening in the 60-cell line assay (months 19-36)

No progress yet.

Key Research Accomplishments

- Improved strategy for the preparation of laulimalide analogs.
- Preparation of subunits **B** and their successful coupling to an appropriate aldehyde (**20**).
- Testing of the first two laulimalide analogs.

Reportable Outcomes

Meeting Presentation

Bradley S. Davidson, "The Microtubule-Stabilizing Agent Laulimalide: Biological Activities And Synthetic Approaches, Thailand Research Fund-RGJ Meeting, Pattaya City, Thailand, April 2003.

Conclusions

During year 3, because of difficulties transferring the solution-phase synthetic route to the solid-phase, work has shifted to modifying the overall strategy and designing a new solid phase linker. Improved substructure **B** fragments were prepared and were demonstrated to couple with an appropriate aldehyde. The first laulimalide analogs prepared in this project were tested for microtubule-stabilizing activity, but were inactive.

References

1. Mooberry, S.L.; Tien, G.; Plubrukarn, A.; Davidson, B.S. Laulimalide and Isolaulimalide, New Paclitaxel-Like Microtubule-Stabilizing Agents. *Cancer Res.* **1999**, *59*, 653.
2. Mulzer, J.; Ohler, E. Microtubule-Stabilizing Marine Metabolite Laulimalide and Its Derivatives: Synthetic Approaches and Antitumor Activity. *Chem. Rev.* **2003**, *103*, 3753.
3. Ghosh, A.K.; Wang, Y.; Kim, J.T. Total Synthesis of Microtubule-Stabilizing Agent (—)-Laulimalide. *J. Org. Chem.* **2001**, *66*, 8973.